Proceedings, Western Section, American Society of Animal Science

Vol. 67, 2016

Effects of rumen protected arginine supplementation to cows during early or late gestation on progeny glucose tolerance[1]

L. R. Owensby[*], C. B. Gardner[*], R. D. Dunlap[†], C. A. Löest[*], S. L. Ivey[*], S. H. Cox[†], A. F. Summers[*] and E. J. Scholljegerdes[*]

[*]Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003

[†]Corona Range and Livestock Research Center, Corona, NM 88318

ABSTRACT: Our hypothesis was calves gestated by dams supplemented rumen protected arginine during early or late gestation would have improved glucose tolerance. In order to test this hypothesis, a two yr study was conducted. Dams were randomly assigned to one of three treatments; 1) grazing native range plus dried distillers grain (Control), or grazing native range plus dried distillers grain and Arg fed to provide 180 mg L-Arg/kg BW either during 2) early gestation (EARG) or 3) late gestation (LARG). In yr 1, 16 yearling calves (heifers n = 8, steers n = 8) and in yr 2, 24 (heifers n = 10, steers n = 14) yearling calves underwent a glucose tolerance test (GTT). On the days of the GTT, cattle were fed at 0600 h and indwelling jugular catheters were inserted at 0700 h. A 50% dextrose solution was injected at 0.5 mL/kg BW via the jugular catheter and subsequent 6 mL blood samples were collected at -5, -2, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, and 120 min relative to the dextrose infusion. Glucose half-lives were estimated by regressing the logarithmically transformed glucose concentrations over time and area under the curve was determined using the trapezoidal summation method. Glucose area under the curve (AUC) did not differ (P = 0.13) between treatment groups; however, overall glucose concentration (conc.) tended (P = 0.06) to be lower for calves of arginine supplemented dams when compared with non-supplemented dams. There were no differences between treatment groups in reference to insulin AUC (P = 0.57), insulin half-life (P = 0.85), or overall insulin concentration (P = 0.47). In conclusion, rumen protected arginine supplementation to cows during varying times in gestation tends to affect overall glucose concentration in progeny during a glucose tolerance test; however, does not affect glucose or insulin AUC, half-life, or overall insulin concentration.

Key words: arginine, glucose AUC, glucose half-life

INTRODUCTION

Poor maternal nutrition can result in increased embryonic mortality rates and potentially generate offspring unable to perform at the level of their non-nutrient restricted counterparts (Barker, 1997; Larson et al., 2009; Funston et al., 2010 a,b). Nutrient restriction can negatively impact size and functionality of the placenta, thereby affecting subsequent blood supply, nutrient and oxygen availability and metabolic efficiency for the conceptus (Vonnahme et al., 2007; Wu et al., 2006). Recent research has indicated that poor placental production of nitric oxide (vasodilatory and angiogenic factor) and polyamines (crucial in DNA and protein synthesis) can contribute to poor fetal development during dam nutrient restriction (Wu et al., 2006). While it is understood that poor maternal nutrition can impact fetal development, the use of supranutritional levels of specific nutrients to enhance development are not well defined.

Arginine, a precursor for the production of nitric oxide and polyamines, may enhance maternal fetal blood exchange through increased placental vascularization. By improving maternal-fetal blood flow, gas, nutrient and waste exchange will also be improved, thus providing maximal nutrients for organogenesis (i.e. pancreatic development). An in vitro study conducted by Rhoten (1980) reported fetal rat pancreata were perinfused with low or high concentrations of glucose in the presence or absence of arginine and leucine. This study demonstrated that fetal rat pancreas development was enhanced when arginine was supplied during gestation (Rhoten, 1980). The hypothesis of this study is calves born to cows supplemented with arginine during gestation will have improved glucose utilization. The main objective is to investigate differences in glucose metabolism of progeny born to dams supplemented arginine during early or late gestation when compared with those born to dams receiving no arginine supplementation.

MATERIALS AND METHODS

All animals and procedures were handled in accordance with the New Mexico State University Institutional Animal Care and Use Committee.

Cow Management and Treatments

A two year study utilized mature Angus × Hereford cows at the Coronal Range and Livestock Research Center (CRLRC) in Corona, NM. All cows were confirmed pregnant to AI 30 d post insemination via blood analysis for pregnancy specific protein B (BioTracking Inc., Moscow, ID). Cows were utilized in a completely randomized design and randomly assigned one of three dietary treatments: 1) grazing native range (average across yr of 95.4% DM, 5.9% CP, 74.18% NDF, DM basis) plus dried distillers grain (**CON**), or grazing native range plus dried distillers grain and rumen protected arginine (55% rumen protection; Miller, 2012) fed to provide 180 mg L-Arg/kg BW either during 2) early gestation (**EARG**; starting on d 55 of gestation) or 3) late gestation (**LARG**; starting on d 210 of gestation). In yr

[1] Research supported in part by the New Mexico Agric. Exp. Stn., Las Cruces, NM.

[2] Corresponding author: ejs@nmsu.edu.

1 (2012) 16 cows (CON n = 6, EARG n = 6, LARG n = 4) were utilized and yr 2 (2013) had 27 cows (Control n = 7, EARG n = 10, LARG n = 10). All cattle were gathered and supplemented individually 3 times per wk for 30 d and the basal diet (DDGs and native range land) was calculated to provide 155% of their arginine requirement (NRC, 2000). A level of 180 mg/kg BW of arginine has been shown to improve vascular hemodynamics in steers (Meyer et al., 2011) and sheep (Saevre et al., 2011). Supplementation windows selected correspond to key points in fetal development that include maximal placental growth, organ differentiation, vascularization and organ growth (Funston et al., 2010a).

Progeny Management

Cows were managed as a single group throughout the experiments at the CRLRC. Following weaning calves were preconditioned for 60 d at the ranch after which calves were transported to New Mexico State University campus in Las Cruces, NM for adaptation to a Calan Gate feeding system (American Calan, Northwood, NH) in order to undergo a subsequent backgrounding trial (yr 1: 56 d and yr 2: 42 d trial) where they were offered ad libitum access to a grower diet (Yr 1 – 19.31% CP, 0.43 NEm, 0.25 NEg; Yr 2 – 10.5% CP, 2.02 NEm, 1.19 NEg).

Glucose Tolerance Test

Following completion of the feed trial, all calves were subjected to a glucose tolerance test (GTT). In each yr, the GTT was conducted over the course of 2 days where steers were tested the first day and heifers were tested the next. On the day of the test, calves were fed at 0600 and catheter placement began at 0700. Following the collection of three baseline samples (time points -5, -3, 0), a 50% dextrose solution was infused at 0.5 mL/kg of BW via the jugular catheter. Blood samples were then collected at time points 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, and 120 min relative to infusion. At each sampling, 7 mL blood was drawn into a sterile syringe that was then dispensed into tubes containing 15 mg of NaF and 12 mg K oxaloate (BD Vacutainer, Franklin Lakes, NJ). Samples were then placed on ice for 30 min and centrifuged at 1,000 x *g* for 20 min at 4°C and plasma was removed and stored at -20°C. Following the collection of the blood sample, 10 mL of saline was inserted into the catheter to clear the catheter until the next blood draw.

Sample analysis

Glucose was analyzed in triplicate using a colorimetric procedure. All plasma samples were diluted by 50% with DI water. Utilizing a 90 well plate, two microliters of standard solutions, controls (BSA), and diluted samples were pipetted into their respective wells. Then 300 microliters of glucose hexokinase was added to each well. The plate was then run using a Synergy H1 Hybrid Plate Reader (BioTek) (Long et al., 2010b). Intra- and inter-assay coefficients of variation averaged 3.84%. Plasma insulin concentrations were determined using a double antibody RIA as described by Sanson and Hallford (1984). Inter-assay CV averaged 8.7% and intra-assay CV average 6.4%.

Calculations and Statistical Analysis

Data were analyzed as a completely randomized design using PROC MIXED (SAS Inst. Inc., Cary, NC). The model included treatment, year, sex and treatment × sex and treatment × year interactions. Glucose half-lives were estimated by regressing the logarithmically transformed glucose concentrations over time. Area under the curve was determined using the trapezoidal summation method as described by Long et al. (2010b). Means were separated using LSMEANS. No interactions were significant ($P \geq 0.22$). A P-value of ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

All glucose tolerance test data is presented in Table 1 and Figure 1 and 2. Prior to glucose infusion, calves born to cows fed arginine during EARG had lower initial blood glucose level ($P = 0.01$) when compared with LARG and CON (Table 1). This is interesting due to the fact that all calves consumed the same diet (39.7% DM, 10.5% CP, 2.02 Mcal/kg NEm, 1.19 Mcal/kg NEg) and dry matter intake did not differ ($P \geq 0.25$) between treatment groups prior to the glucose challenge. Overall glucose concentrations did not differ between groups ($P = 0.14$); however, numerically, EARG calves had lower overall glucose concentration. Calves from arginine supplemented dams (EARG and LARG) tended to have lower overall blood glucose concentrations ($P = 0.06$) compared to CON. In yr 1, glucose area under the curve (AUC) tended to be lower ($P = 0.06$) for the EARG group and those calves in the arginine groups (EARG and LARG) tended to have a smaller glucose AUC ($P = 0.11$); however, no differences were found in glucose AUC in yr 2 calves ($P = 0.55$). When yr 1 and 2 data were combined, no differences for glucose AUC ($P = 0.13$) were observed between treatments, with only numerical differences being observed, with EARG having numerically lower glucose AUC. Further, glucose half-life did not differ between treatment groups ($P = 0.95$). Insulin AUC, half-life, and overall concentration did not differ ($P = 0.57$; $P = 0.85$; $P = 0.78$, respectively). Insulin AUC was different ($P = 0.0008$) between yr 1 and yr 2 calves; however, no other sex, year, treatment x sex or treatment x year interactions differed ($P \geq 0.43$ and $P \geq 0.45$, respectively) for any other measurements.

Due to our current small sample size, only numerical differences were observed, nevertheless our results do agree with previous data (Ford et al., 2007; Long et al., 2010a; Zhang, 2010) where it was found that maternal nutrition can affect offspring's ability to metabolize glucose. Ford et al., (2007) found that lambs born to nutrient restricted ewes exhibited a significantly larger glucose AUC ($P < 0.05$) and insulin AUC ($P < 0.001$) as determined during a glucose tolerance test when compared to lambs born to non-restricted ewes at d 63 of age. Furthermore, lambs of nutrient restricted dams displayed a larger glucose AUC ($P < 0.01$), but a smaller insulin AUC ($P = 0.05$; Ford et al., 2007). Our original overarching hypothesis is that arginine supplementation during early gestation will affect the development of key organs such as the pancreas during fetal development. Numerical differences and near tendencies reported in the present study suggest calves born to arginine supplemented dams may have a increased ability to absorb glucose from the blood into the tissues. This may indicate that arginine supplementation, especially during early gestation, is beneficial to pancreatic development and maturation during fetal development. However, other mechanisms involving the insulin dependent glucose

transporter, GLUT 4, may play a role. For example, Long et al. (2010c) found that steers born to nutrient restricted dams displayed a decreased amount of GLUT 4 in the peri-renal adipose tissue (Gardner et al., 2005; Long et al., 2010c); however, contradicting results were found in a separate study where calves born to cows on a low-plane of nutrition had an increased time to plasma glucose clearance (Long et al., 2010b). The results obtained thus far indicate arginine supplementation during gestation may influence offspring glucose metabolism; however, the mechanism behind this observation has yet to be determined. Therefore, current work is being conducted to examine pancreatic tissues from calves to determine differences in Islet of Langerhans numbers, insulin secretion by pancreatic beta cells, and pancreatic insulin.

Table 1. The effects of maternal Arg supplementation during early or late gestation on glucose and insulin area under the curve (AUC) and glucose half-life after glucose tolerance test

	Treatments[1]				
Item	CON	EARG	LARG	SE	*P*-value
n	13	15	12		
Glucose					
AUC[2]	518.4	499.9	520.4	8.3	0.13
Half - life, min[3]	102.6	99.7	104.5	11.0	0.94
Initial conc., mg/dL	68.4[a]	55.8[b]	68.9[a]	3.8	0.01
Overall conc., mg/dL	108.6	99.6	102.5	3.4	0.14
Insulin					
AUC[2]	742.4	727.9	725.8	23.3	0.85
Half -life, min[3]	90.1	76.8	96.1	16.9	0.61
Overall conc., ng/dL	6.2	1.8	7.9	4.1	0.47

[a-b]Means with different superscripts differ $P \leq 0.05$.

[1] Dams grazed native range and were provided dried distillers grain (CON), or grazing native range plus dried distillers grain and rumen protected arginine (55% rumen protection) fed to provide 180 mg L-Arg/kg BW either during early gestation (EARG; starting on d 55 of gestation) or late gestation (LARG; starting on d 210 of gestation).

[2]Area under the curve was determined using the trapezoidal summation method.

[3]Half-life was calculated as the time required for a 50% decrease in peak plasma glucose and insulin concentration.

LITERATURE CITED

Barker, D. J. P. 1997. Maternal nutrition, fetal nutrition, and disease in later life. Nutr. 13:807-813.

Ford, S. P., B. W. Hess, M. M. Schwope, M. J. Nijlan, J. S. Gilbert, K. A. Vonnahme, W. J. Means, H. Han, and P. W. Nathanielsz. 2007. Maternal undernutrition during early gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. J. Anim. Sci. 85:1285-1294. doi:10.2527/jas.2005-624

Funston, R. N., D. M. Larson, and K. A. Vonnahme. 2010a. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production. J. Anim. Sci. 88:E205-E215. doi:10.2527/jas.2009-2351

Funston, R. N., J. L. Martin, D. C. Adams, and D. M. Larson 2010b. Winter grazing system and supplementation of beef cows during late gestation influence heifer progeny. J. Anim. Sci. 88:4094-4101. doi:10.2527/jas.2010-3039

Gardner, C. B. 2014. Effect of rumen protected arginine supplementation on livestock performance. Thesis. New Mexico State Univ., Las Cruces, NM.

Gardner, D. S., K. Tingey, B. W. M. Van Bon, S. E. Ozanne, V. Wilson, J. Dandrea, D. H. Keisler, T. Stephenson, and M. E. Symonds. 2005. Programming of glucose-insulin metabolism in adult sheep after maternal nutrition. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289:R947-R954. doi:10.1152/ajpregu.00120.2005

Larson, D. M., J. L. Martin, D. C. Adams, and R. N. Funston. 2009. Winter grazing system and supplementation during late gestation influence performance of beef cows and steer progeny. J. Anim. Sci. 87:1147-1155. doi:10.2527/jas.2008-1323

Long, N. M., L. A. George, A. B. Uthlaut, D. T. Smith, M. J. Nijland, P. W. Nathanielsz, and S. P. Ford. 2010a. Maternal obesity and increased nutrient intake before and during gestation in the ewe results in altered growth, adiposity, and glucose tolerance in adult offspring. J. Anim. Sci. 88:3546-3553. doi:10.2527/jas.2010-3083.

Long, N. M., M. J. Prado-Cooper, C. R. Krehbiel, and R. P. Wettemann. 2010c. Effects of nutrient restriction of bovine dams during early gestation on postnatal growth and regulation of plasma glucose. J. Anim. Sci. 88: 3262-3268. doi:10.2527/jas.2010-3214

Long, N. M., M. J. Prado-Cooper, C. R. Krehbiel, U. DeSilva, and R. P. Wettemann. 2010b. Effects of prenatal nutrient restriction of bovine dams during early gestation on postnatal growth, carcass and organ characteristics, and gene expression in adipose tissue and muscle. J. Anim. Sci. 88:3251-3261. doi:10.2527/jas.2009-2512

Meyer, A. M., C. B. Saevre, D. V. Dhuyvetter, R. E. Musser, and J. S. Caton. 2011. Effects of rumen protected arginine supplementation on serum amino acid concentrations in forage-fed steer. Proc. West Sect. Am. Soc. Anim. Sci. 62:368-371.

Miller, N. P. 2012. Utilization of dried distillers grains to manage rate of body weight gain during heifer development and the use of a novel rumen-protected arginine to alter plasma levels of arginine. M.S. Thesis, New Mexico State University, Las Cruces.

Rhoten, W. B. 1980. Insulin secretory dynamics during development of rat pancreas. Am. J. Physiol. 239:E57-E63.

Saevre, C. B., J. S. Caton, J. S. Luther, A. M. Meyer, D. V. Dhuyvetter, R. E. Musser, J. D. Kirsch, M. Kapphahn, D. A. Redmer, and C. S. Schauer. 2011.

Effects of rumen-protected arginine supplement on ewe serum amino acid concentration, circulating progesterone, and ovarian blood flow. Sheep and Goat Res. 26:8-12.

Sanson, D. W. and D. M. Hallford. 1984. Growth response, carcass characteristics, and serum glucose and insulin in lambs fed tolazamide. Nutr. Rep. Int. 29:461.

Vonnahme, K. A., M. J. Zhu, P. P. Borowicz, T. W. Geary, B. W. Hess, L. P. Reynolds, J. S. Caton, W. J. Means, and S. P. Ford. 2007. Effect of early gestation undernutrition on angiogenic factor expression and vascularity in the bovine placentome. J. Anim. Sci. 85:2464-2472. doi: 10.2527/jas.2006-805

Wu G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Board-invited review: intrauterine growth retardation: implications for the animal sciences. J. Anim. Sci. 84:2316-2337. doi:10.2527/jas.2006-156

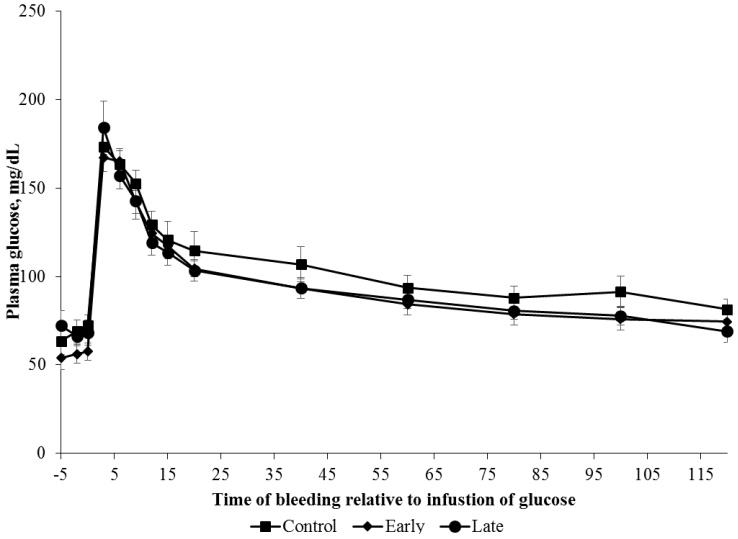

Figure 1. The effects of maternal Arg supplementation during early or late gestation on progeny plasma glucose concentrations after performing a glucose tolerance test. Effects for the entire bleeding period included: treatment ($P = 0.28$), time ($P < 0.0001$), treatment × time ($P = 0.58$), sex ($P = 0.64$), treatment x sex ($P = 0.11$), and year ($P = 0.04$).

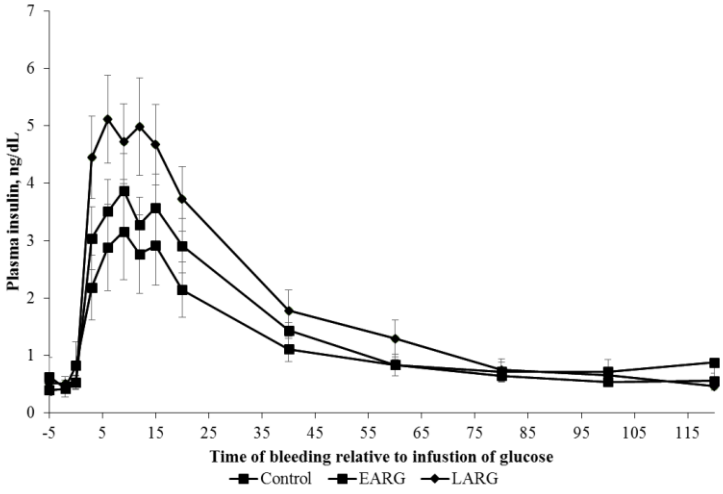

Figure 2. The effects of maternal Arg supplementation during early or late gestation on progeny plasma insulin concentrations after performing a glucose tolerance test. Effects for the entire bleeding period included: treatment ($P = 0.47$), time ($P = 0.02$), treatment x time ($P = 0.98$), sex ($P = 0.15$), treatment x sex ($P = 0.57$), year ($P = 0.12$) and treatment x year ($P =$